BSC Research Concept Review

Meeting of the National Toxicology Program
Board of Scientific Counselors

National Institute of Environmental Health Sciences
Rall Building, Rodbell Conference Center
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NTP Study Nomination: Aminopyridines

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1. Is a clear and valid rationale for the proposed research program articulated in the NTP research concept document?

The rationale for testing 2-, 3-, and 4-aminopyridine presented in the summary document were several fold.

(1) There is a lack of standard toxicity studies for the monoaminopyridines. Toxicity of 4-AP has been previously reviewed (Schafer et al., 1973), but these data are quite old and certainly not up to current testing standards, let alone under GLP compliance.

(2) 2-aminopyridine and 3-aminopyridine are used as chemical intermediates in the production of pharmaceuticals. Production volumes for 2-aminopyridine are moderate and the volumes for 3-aminopyridine are unknown.

(3) 4-aminopyridine is currently used as a pesticide and is in development as a therapeutic to treat multiple sclerosis. In particular, the use of 4-AP as a human pharmaceutical indicates potential significant (intentional) human exposure. There have been a number of reported cases of human toxicity, usually following overdose with 4-AP taken as a pharmaceutical. Curiously, these were not cited in the Research Concept document. In addition, the document suggests that 4-AP is being proposed for human use, and there is a mention of a 2007 FDA clinical trial approval, yet 4-AP has had reported human exposure as far back as the early 1990s (Stork and Hoffman, 1994). Other human reports of 4-AP toxicity have also been reported (Pickett and Enns, 1996; Johnson and Morgan, 2006) and these effects have typically included cardiovascular and neurologic effects of 4-AP.

(4) The combined testing of these three monoaminopyridines will contribute to a better understanding of the structure activity relationships within this chemical class.

Although these rationale provide adequate justification for testing by the NTP, there are additional reasons to test this series of chemicals that are not specifically stated in the research concept document. First, as pointed out in the background material, the limited number of workers exposed to the chemical based on production volumes and reporting criteria under the PAIR rule do not take into account downstream uses of the material that provide additional human exposure. Second, the advancement of 4-aminopyridine into clinical trials for multiple sclerosis will require completion of the standard battery of toxicological tests required by the FDA including an eventual rodent cancer bioassay (assuming the drug will be taken for more than 6 months which seems likely for multiple sclerosis). Although the pharmaceutical sponsor is not required to make public any toxicology data prior to drug approval, it would be worthwhile to contact the sponsor and request the data associated with 4-aminopyridine which would allow the NTP and NCI to leverage these studies in the structure activity analysis.

Given the known human adverse effects, the large production volumes, and lack of useful data, study of the toxicity of these compounds is warranted.

2. Is the proposed research program as outlined in the research concept document appropriate in scope given the public health importance of the issue or substance proposed for study? Are there other studies that should be considered as part of this research program?

The research concept, in general seems appropriate, though specific details have not been provided on the exact nature of the test programs proposed.

The specific aims outlined in the research concept document are not identical to the data gaps identified by the NCI in the document supporting their nomination. In the NCI document, the suggested studies include a rodent cancer bioassay on only 2-aminopyridine while 3- and 4-aminopryidine would be assessed using only short-term mechanistic studies. In addition, supporting neurotoxicity studies were suggested to be performed as needed to compare among the monoaminopyridines. In the research concept document, only a brief description of the three specific aims is provided. It is not explicitly stated, but the first specific aim implies that all three monoaminopyridines will be tested in toxicity studies. It is assumed that these are the standard two week and thirteen week studies and the results of these studies will determine which monoaminopyridines will be selected for chronic testing.

The second specific aim describes pharmacokinetic and metabolism studies to characterize the distribution and excretion of the parent chemical and various metabolites. The third and last specific aim outlines genomic, genotoxicity, and high-throughput screening tests. Although the toxicity tests and pharmacokinetic studies may be relatively standardized at the NTP, there are no standard approaches for genomic and high-throughput screening assays in toxicology and a more detailed description of the study design would have been beneficial. If characterizing the mode-of-action of target organ toxicity is the primary goal of the genomic studies, the project leader is strongly encouraged to consider

performing the studies in dose response and at multiple time points. The inclusion of additional positive control chemicals would also aid in interpreting the data. These could include other potassium channel blockers for cardiotoxicity and phenobarbital-like inducers for liver toxicity given the Cyp2b1 induction and the cross-species tumor profile in the pyridine bioassay. To assess the potential relevance of the mode-of-action to humans, measurements of orthologous gene expression changes in *ex vivo* tissue culture models should also be considered (e.g., primary cells, tissue slices, etc.). It should be noted that the design of the microarrays is critical in interpreting cross-species changes in expression (Xing *et al.*, Mol. Biol. Evol. 24:1283, 2007). Finally, the rationale for including high-throughput assays in assessing the toxicity of the monoaminopyridines is a bit confusing since the characterization of only three aminopyridines does not require high-throughput methods. It is unclear whether the three aminopyridines will be added to the existing NTP high-throughput screening initiative, whether specific ion channel assays from the initiative will be employed to evaluate the three aminopyridines, or whether additional members from the chemical class would also be evaluated.

Neurotoxicity and cardiovascular effects should be a primary focus area as these are the primary target organs implicated in human exposures to 4-AP and are consistent with the known mechanism of K^+ channel blocking of the aminopyridines. The proposed in vitro electrophysiology studies are recommended to be placed in a lower priority. These studies will provide little additional data that will not be better studied in direct in vivo nerotoxicity assessments.

Although immunotoxicity is mentioned as an endpoint, it is not stated whether the standard panel of immunomodulation tests will be performed. It is recommended by reviewers to place immunotoxicity studies in a lower priority in this program. While some mechanistic data suggests a possible immunologic effects of APs, this can be assessed as part of the routine repeat-dose toxicity studies. If no obvious immunologic target organs are identified in repeat-dose studies, the need for additional supplemental immunotoxicity studies may not be warranted.

3. Does the proposed research program address an important area of biomedical research (e.g. children's health, genetic susceptibility, specific environmental disease) and/or advance the field of environmental health sciences?

This program does not specifically address children's health, genetic susceptibility, or specific environmental disease, and APs cannot be considered a major environmental contaminant. Nevertheless, their broad use and, in particular, their use in humans for various orphan disease indications suggests they are of sufficient importance to warrant study.

Reviewers recommend that before embarking on animal toxicity studies, NIEHS exert some effort to obtain toxicology reports that have been submitted to the FDA as part of presumably approved previous IND applications for 4-AP. If these studies are conducted with a reasonable degree of scientific rigor and regulatory compliance, the need for additional testing, and certainly for dose rangefinding studies, may be reduced or eliminated,

at least for 4-AP, and these results may guide dose rangefinding study design for 2- and 3-AP.

The inclusion of genomic measurements by the NTP in a formal testing design represents a significant step forward for the field of environmental health sciences as the technology transitions from a research application to a testing and regulatory application. Given the implications of this transition, it would seem prudent that the study designs of the initial chemicals utilizing these approaches should be carefully scrutinized and subject to further review by an independent advisory panel to maximize the information gained from these studies and promote acceptance by the various stakeholders. Lessons learned by the FDA on the submission, evaluation, and interpretation of genomic data in preclinical toxicity studies should also be leveraged.

4. Does the proposed research program merit utilization of NTP resources, and if so, what priority (low, moderate, or high) should it be given?

As noted above, the APs have a large enough human exposure and production use that they warrant study. Reviewers divided on priority, with one (Thomas) giving this high priority and another (Mirsalis), placing this as moderate priority; however, both considered this appropriate for utilization of NTP resources.

References Cited

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